CHAPTER 8

General Discussion and Future Perspectives

Cardiovascular disease (CVD) is the leading cause of death worldwide despite the successful development of several pharmaceutical interventions of which statin therapy is the dominating lipid-lowering treatment option.[1] However, the treatment of CVD remains suboptimal due to; (i) a residual risk that persists after statin treatment,[2] (ii) failure for some patients to reach low-density lipoprotein-cholesterol (LDL-C) targets despite statin treatment,[3] and (iii) lack of adherence to statin treatment as a result of amongst others statin intolerance.[4] Atherosclerosis, a chronic inflammatory disease of multifactorial origin,[5, 6] is a dominant contributor to the development of CVD.[7] The research described in this thesis investigated the effects of innovative pharmaceutical interventions in experimental atherosclerosis, targeting hypertension and high blood cholesterol, more specifically high LDL-C and low high-density lipoprotein-cholesterol (HDL-C), as risk factors for CVD. We used APOE*3Leiden.CETP mice which express human cholesteryl ester transfer protein (CETP) under control of its natural flanking regions.[8] These mice have impaired clearance of apolipoprotein B-containing lipoproteins and mimic the slow clearance observed in humans, particularly in patients with familial dysbetalipoproteinemia (FD).[9] The APOE*3Leiden.CETP mouse model is a well-established model for lipid and lipoprotein metabolism and atherosclerosis, because these mice; (i) develop diet-induced atherosclerosis, (ii) have a human-like lipoprotein metabolism and, (iii) respond in a human-like manner to lipid-modifying treatment strategies, including LDL-C-lowering and HDL-C-raising compounds.[10-15]

In view of the fact that hypertension is a leading risk factor for CVD and associated with the development of atherosclerosis,[16, 17] we investigated the anti-atherosclerotic effects of aliskiren, the first commercially available, orally active, direct renin inhibitor approved for the treatment of hypertension in **chapter 2**.[18] In this study in APOE*3Leiden.CETP mice, we demonstrated beneficial effects of aliskiren on atherosclerosis development and plaque stability when administered alone and in combination with atorvastatin. Aliskiren reduced systolic blood pressure and additionally reduced atherosclerotic lesion size and severity. Interestingly, the reduction in atherosclerosis development observed by aliskiren remained after correcting for blood pressure, suggesting that aliskiren had anti-atherosclerotic properties beyond its blood pressure-lowering qualities. Aliskiren also improved plaque stability as evidenced by a decrease in macrophage and necrotic area, as well as by an increase in SMC content in the cap, possibly via a mechanism involving T cells. The combination of aliskiren and atorvastatin was more potent in reducing atherosclerotic lesion size, as well as markers of inflammation and in improving plaque stability. Clinical trials, including the ALLAY (Aliskiren in Left-Ventricular Hypertrophy),[19] the ALOFT (Aliskiren Observation of Heart Failure Treatment)[20] and the AVOID (Aliskiren in the Evaluation of Proteinuria In Diabetes) trials[21] reported beneficial effects of aliskiren on various markers of organ damage. However, aliskiren in combination with angiotensin converting enzyme inhibitors (ACEi) and angiotensin II type I receptor blockers (ARBs) failed to provide additional

cardiovascular benefit in diabetic patients at high risk of developing cardiovascular and renal complications in the ALTITUDE trial[22] and in patients hospitalized for heart failure in the ASTRONAUT trial.[23] Both these trials reported more adverse events, i.e. renal dysfunction, hyperkalemia and hypotension. In contrast, results from the prematurely terminated APOLLO trial that investigated the cardiovascular protective effects of aliskiren monotreatment and in combination with hydrochlorothiazide and amlodipine in elderly patients[24] and the AQUARIUS trial that evaluated the effects of aliskiren on coronary atherosclerosis in patients with prehypertension, revealed potential for CVD reduction.[25] The latter trial reported a non-significant trend towards a reduction in atheroma volume from baseline after aliskiren treatment. The ongoing ATMOSPHERE trial (NCT00853658) evaluating the efficacy and safety of aliskiren and aliskiren + enalapril combination treatment in patients with chronic heart failure will provide additional insight into the protective role of aliskiren and results are expected in 2015.

Cholesterol contained in LDL particles is well recognized as a primary causal risk factor for coronary heart disease (CHD) as evidenced by experimental, epidemiological and genetic data.[26] Furthermore, intervention trials provided ample evidence that the lowering of LDL-C contributes to a reduction in CHD.[27-29] However, despite the fact that epidemiological studies consistently reported an inverse association between HDL-C and CHD risk,[30-32] the benefits of raising HDL-C remain less defined. In Chapter 3 to 6, we investigated the effects of novel lipid-modifying treatment strategies, i.e. LDL-C-lowering and/or HDL-C-raising compounds on atherosclerosis development in the APOE*3Leiden.CETP mouse model, since these mice respond to both LDL-C-lowering and HDL-C-raising compounds in a human-like manner.

The benefits of niacin on plasma lipids were first described in 1955 and led to the development of niacin for therapeutic purposes.[33] In **chapter 3**,[34] we aimed to address the discrepancy between the beneficial effects of niacin in initial clinical trials[35-38] and the lack of effect of niacin on top of statin treatment on the reduction of CVD events in the large AIM-HIGH and HPS2-THRIVE clinical trials[39, 40] by evaluating the effects of niacin alone and in combination with simvastatin on plasma lipid levels and atherosclerotic lesion size and composition. We demonstrated that niacin reduced (V)LDL-C and (V)LDL-TG and that the increase in HDL-C may be attributable to a decrease in hepatic and plasma CETP. Importantly, the extent of lipid-lowering observed with niacin in our study in E3L.CETP mice was comparable to that of FD patients.[34, 41, 42] Moreover, we showed that niacin decreased atherosclerosis development mainly by reducing non-HDL-C with a modest HDL-C-raising and additional anti-inflammatory effects. We demonstrated that the additive effect of niacin on top of simvastatin was mostly dependent on its non-HDL-C-lowering capacities. Based on these findings and results from a reverse cholesterol transport (RCT) experiment, we conclude that the effects of niacin on HDL-C and HDL functionality may partially contribute to, but is not the driving force behind its anti-atherogenic effects observed in our study.

Therefore, data from our study suggested that clinical beneficial effects of niacin are largely dependent on its ability to lower (V)LDL-C on top of concomitant lipid-lowering therapy and may explain the failure of niacin in the AIM-HIGH and HPS2-THRIVE trials in hyperlipidemic patients subjected to aggressive LDL-C-lowering treatment with limited effects of niacin on (V)LDL-C.[39, 40]

In 1989, markedly increased HDL-C led to the discovery of the first mutation in the CETP gene in two Japanese subjects.[43] CETP facilitates the transfer of cholesteryl esters from atheroprotective HDL to atherogenic V(LDL) and has become a target to increase HDL-C. This has led to the development of several small molecule CETP inhibitors, including amongst others torcetrapib, dalcetrapib, anacetrapib and evacetrapib. However, despite favorable effects on both LDL-C and HDL-C, torcetrapib increased mortality in the large phase III ILLUMINATE trial, most likely due to an off-target increase in aldosterone which leads to activation of RAAS and an increase in blood pressure,[44] potentially resulting in a more vulnerable plaque phenotype.[13] In the large phase III dal-OUTCOMES trial, dalcetrapib which only raises HDL-C without affecting LDL-C had no effect on cardiovascular events in patients with recent acute coronary syndrome (ACS) and although not significant, the 0.6 mmHg rise in systolic blood pressure and 18% increase in C-reactive protein certainly warrants attention, specifically with regards to other CETP inhibitors that are currently in clinical development.[45] In **chapter 4**,[46] we investigated the effects of a broad dose range of the novel CETP inhibitor, anacetrapib on CETP activity, lipid levels, atherosclerotic lesion size and composition, as well as HDL function and we examined possible additive/synergistic effects of anacetrapib on top of atorvastatin. In our study, anacetrapib dose-dependently reduced CETP activity, thereby decreasing non-HDL-C and increasing HDL-C. These lipid-altering effects were comparable to findings from clinical trials.[47-49] Moreover, anacetrapib dose-dependently reduced atherosclerosis development. This effect was mainly ascribed to a reduction in non-HDL-C despite a remarkable increase in HDL-C and without affecting HDL functionality. Interestingly, anacetrapib itself also contributed to the reduction of the lesion size by a hitherto unknown mechanism. In addition, anacetrapib improved lesion stability when given at a higher dose and a moderate dose anacetrapib added to the anti-atherogenic effects of atorvastatin. In phase II clinical trials, neither anacetrapib nor evacetrapib showed the side effects observed with torcetrapib treatment and both compounds were more potent in reducing LDL-C and increasing HDL-C when compared to torcetrapib and dalcetrapib.[49-51] The effects of anacetrapib and evacetrapib on clinical outcomes are currently being investigated in the large phase III REVEAL (NCT01252953) and ACCELERATE (NCT01687998) clinical trials and results are expected in 2016/2017. We further explored the mechanism by which anacetrapib reduces (V)LDL-C and whether this effect is dependent on the inhibition of CETP activity in **chapter 5**. In this study, we showed that anacetrapib reduces (V)LDL-C by increasing hepatic remnant uptake via two

mechanisms; (i) inhibition of CETP activity, resulting in remodelled VLDL particles that are more susceptible to hepatic uptake, and (ii) a CETP-independent reduction in plasma proprotein convertase subtilisin/kexin type 9 (PCSK9) level that has the potential to increase LDL receptor (LDLR)-mediated hepatic remnant clearance. According to our knowledge, there are currently no clinical data describing the mechanism by which anacetrapib reduces non-HDL-C/LDL-C. However, a reduction in plasma PCSK9 levels after anacetrapib treatment was in accordance with findings in rhesus macaques[52] and results from our study confirmed a CETP-independent decrease in plasma PCSK9 levels by anacetrapib as previously observed in C57BL/6 mice.[53]

In 2003, Abifadel *et al.* identified two French families with autosomal dominant hypercholesterolemia caused by mutations in PCSK9.[54] PCSK9 is a serine protease responsible for LDLR degradation by preventing the recycling of the receptor to the cell membrane after internalization.[55] The upregulation of the LDLR after statin treatment is accompanied by an upregulation of PCSK9 which in turn promotes LDLR degradation.[56-58] Inhibition of PCSK9 is, therefore, a potential novel strategy in the treatment against CVD, especially in combination with statin treatment. We, therefore investigated the effects of 2 dosages of the fully human, monoclonal antibody against PCSK9, alirocumab alone and in combination with atorvastatin on hepatic LDLR protein levels and hepatic cholesterol metabolism, plasma lipid levels, atherosclerosis development and plaque morphology in **chapter 6**.[59] In this study, alirocumab dose-dependently increased hepatic LDLR protein levels without changes in hepatic cholesterol and TG levels and consequently decreased plasma cholesterol levels and reduced the development of atherosclerosis. Moreover, alirocumab improved lesion morphology and composition and enhanced the beneficial effects of a mild dose of atorvastatin. The anti-atherosclerotic effect was strongly dependent on the reduction of plasma TC levels, indicating that the majority of the effect was brought about by cholesterol-lowering leaving limited/no space for other potential (pleiotropic) effects. This is the first study to show that a monoclonal antibody to PCSK9 reduces atherosclerosis development. It should be noted that this is a progression/prevention study which may pose as a potential limitation with respect to translation to the clinic where patients with existing lesions are often treated. Nonetheless, data from this study may also suggest beneficial effects on markers of atherosclerosis by reducing TC with alirocumab in the human situation where new lesions are formed alongside existing plaques. The dose-dependent cholesterol-lowering effects observed in our study were in accordance with results from phase I and II clinical trials.[60-62] No significant safety issues emerged from these short term trials. Results from phase III trials within the ODYSSEY programs will provide further insight regarding the long term efficacy, safety and tolerability of alirocumab in patients with familial hypercholesterolemia and in high CVD risk patients with hypercholesterolemia on lipid-modifying therapy.[63] The large ODYSSEY Outcomes trial (NCT01663402) evaluating the effects of alirocumab on the

occurrence of cardiovascular events in patients with relatively recent ACS treated with high-dose statins will reveal whether PCSK9 inhibition with alirocumab translates into clinical benefit and results are expected in 2018.

In **chapter 7**, we reviewed the effects of established and novel treatment strategies, specifically targeting HDL, other than statins on inhibition of atherosclerosis development in preclinical studies in animals expressing CETP, a crucial gene involved in HDL metabolism and implicated in the mechanisms by which most therapies modulate HDL.[64] In addition, we conducted a meta-analysis to evaluate the potential effects of these treatment strategies on the prevention of clinical events in randomized controlled trials. In the systematic review and meta-analysis, we focused specifically on the contribution of non-HDL-C/LDL-C-lowering versus HDL-C-raising on inhibition of atherosclerosis and the prevention of CVD. Using data from relevant preclinical studies, we found significant correlations between both TC and non-HDL-C exposure and atherosclerosis, however, there was no significant association between HDL-C exposure and atherosclerosis. The meta-analysis of relevant clinical trials revealed no association between the absolute or percentage increase in HDL-C and non-fatal myocardial infarction, whereas a trend toward an association between the absolute decrease in LDL-C levels and non-fatal myocardial infarction was found. This is in line with results from a recent meta-analysis involving only statin trials (8 trials with 38 153 participants), showing that HDL-C and apolipoprotein A-I levels, as well as the increase in apolipoprotein A-I were associated with reduced cardiovascular risk, however no association was found for the increase in HDL-C.[65] The effects of other novel treatment strategies specifically targeting HDL, including reconstituted and delipidated HDL, as well as HDL mimetics, apolipoprotein A-I mimetic peptides, recombinant apolipoprotein A-I Milano and recombinant human lecithin cholesterol acyltransferase (LCAT) seem promising in the protection against atherosclerosis development and cardiovascular disease based on data from preclinical studies[66-77] and clinical trials.[78-81] In these studies, the lack of plasma lipid modification suggests a direct role of HDL and/or apolipoprotein A-I, possibly via an increase in reverse cholesterol transport and supports the view that HDL function rather than HDL-C may have a causal relation to atherprotection.[82]

Thus, according to results from our systematic review and meta-analysis, as well as supporting evidence obtained from the literature, it is evident that the protective role of lowering LDL-C and non-HDL-C is well-established, although occasionally LDL-C lowering compounds have failed due to (off-target) side effects. The contribution of raising HDL-C on inhibition of atherosclerosis and the prevention of cardiovascular disease remains undefined and may be dependent on the mode of action of HDL-C-modification. Nonetheless, treatment strategies aimed at improving HDL function and raising apolipoprotein A-I may be worth exploring.

In conclusion, the research described in this thesis provides evidence for anti-atherogenic effects of several innovative pharmaceutical interventions that are currently being investigated in clinical trials, specifically targeting hypertension and hypercholesterolemia as risk factors for CVD. Our results further support additional benefit of these treatment strategies in combination with statin treatment which is currently the 'gold standard' therapy for the treatment of CVD. Most of these lipid-modifying treatment strategies affect both LDL-C and HDL-C and we demonstrate that the beneficial effects of these treatment strategies predominantly derive from their non-HDL-C/LDL-C-lowering abilities. Nonetheless, results from preclinical studies and clinical trials support the notion that treatment strategies aimed at improving HDL function and raising apolipoprotein A-I may also inhibit the development of atherosclerosis and reduce the prevalence of CVD.

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